IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant:	SAKAI, Takuo	Conf.:	7643
Appl. No.:	10/069,182	Group:	1651
Filed:	May 22, 2002	Examiner:	Ware, Deborah K.
For:	PROCESS FOR PRODUCING PLANT-ORIGIN ANTIBACTERIAL SUBSTANCE		

DECLARATION UNDER 37 CFR §1.132

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

I, Mr. Takuo SAKAI, declare the following:

I am the inventor of the invention described in the above-identified application. I am fully knowledgeable of the disclosure of the above-identified application and the field of art of the present invention. I have read and understand the Office Action dated March 23, 2004 and the references cited therein; WO 01/07135 (WO '135), U.S. Patent No. 6,063,382 (US '382), EP 0880894 (EP '894), and Sakai et al. (*Agric. Biol. Chem.*, Vol. 54, No. 4, pages 879-889, 1990).

A) 1. I am a citizen of Japan residing at Sakai-shi, Osaka, JAPAN;

2. (a) I received a doctor's degree in 1965 from the Graduate School of Agriculture, Kyoto University, Japan;

(b) I was employed by TANABE SEIYAKU CO., LTD. in 1965, where as a research worker in 1965-1970, I was involved in research work

on production of functional sacharides (fructose 1,6-bisphosphate) and research work on production of coenzyme type nucleotide.

(c) From 1970 to 1990, I served as associate professor in the Faculty of Agriculture, Osaka University, Japan, where I was involved in research work on utilization of ligneous particles for the food circulatory system, research work on biological wrought of cotton fiber, research work on ume vinegar and research work on protopectinase enzyme. Papers about my research work on protopectinase enzyme were published in the Journal of Agricultural and Biological Chemistry in 1988, 1989, 1990, etc.

(d) From 1997 to 2003, I served as professor in the Faculty of Agriculture, Kinki University, Japan. In 2003, I founded IGA Bio Research, a limited company.

B) The present invention relates to a process of producing an antibacterial substance derived from a plant which includes disintegrating at least a part of the tissue of the plant and releasing the antibacterial substance therefrom, and bactericidal or bacteriostatic compositions containing as an active ingredient the antibacterial substance obtained by the process. The uniqueness of the present invention lies in the fact that the antibacterial substance is obtained from the tissue of the plant by disintegrating the plant tissue with an enzyme (e.g., protopectinase) capable of acting on protopectin

to release a pectin substance.

The following experiments were performed by me or under my direct supervision. It is clear from the data obtained from these experiments that the means of disintegrating plant tissue has a profound effect on the bactericidal or bacteriostatic properties of the resulting composition.

The below experimental data shows that when comparing an antibacterial substance produced using the protopectinase of the present invention (herein referred to as Sample (A)) and an antibacterial substance produced from the same plants as used for Sample (A) without using the protopectinase (herein referred to as Sample (B)), that Sample (A) is superior to Sample (B) in the effect of inhibiting germination of spores as shown in TABLE 1 below.

An experiment is carried out as follows:

Materials: cabbage, lettuce, potato, pumpkin and Chinese cabbage.

Bacteria examined on budding of their spores: Bacillus subtilis 6633.

Bacteria examined on their growth: Bacillus subtilis IF03134 strain.

Methods for preparing samples:

(i) Method for preparing the antibacterial substance of the present invention:

A material (15g) is chopped into 0.5 cm squares and suspended in 30 ml of 100 mM Tris-HCl buffer (pH 7.0). Protopectinase (0.2 g) is added to the resultant suspension and the mixture is reacted at 37 °C for two hours. After the reaction, the resulting solution is centrifuged to remove the solid from the solution, thereby giving a supernatant liquid as sample (A).

(ii) Method for preparing the antibacterial substances of the prior art:

A material (15g) is chopped into 0.5 cm squares and suspended in 30 ml of 100 mM Tris-HCl buffer (pH 7.0), and then were sufficiently grounded by a homogenizer made of glass. The resulting solution is centrifuged to remove the solid from the solution, thereby giving a supernatant liquid as sample (B).

The number of spores, which have budded and the number of grown cells have been calculated according to the methods described in the present specification using a 100 μL amount of sample added.

The number of spores and the number of grown cells can be calculated by the following method:

First, a PABS solution (0.1 to 0.5 ml) is added to 0.9 ml of a GYP medium, and 5 minutes after the addition, 0.1 ml of spores or cells (100,000 spores or cells per ml) of B. subtilis is added to the resulting solution, and then, the thus obtained solution is allowed to stand at 5℃ for 2 hours. Next, the resulting solution (0.05 ml) is plated onto an agar GYP plate medium (with an agar content of 1.5%) and cultivated at 37℃ for 48 hours, and then the number of colonies appearing is counted.

TABLE 1

Samples	(1) Number of spores having germed	(2) Number of grown cells (colonies/ml)
Water (control)	560	620
Lettuce (A)	51	70
Lettuce (B)	108	72
Cabbage (A)	82	156
Cabbage (B)	183	235
Potato (A)	260	620
Potato (B)	385	620
Pumpkin (A)	55	285
Pumpkin (B)	128	542
Chinese cabbage (A)	48	230
Chinese cabbage (B)	253	255

From the fact that Sample(A) and Sample(B) show different effects, I firmly believe these are different substances though they are made from the same plant material. Furthermore, I firmly believe that the skilled artisan would not find the inventive process as defined in claim 1, as presently amended,

nor the product obtained from the inventive process as defined in claim 7 obvious over the cited prior art.

C) The undersigned hereby declares that all statements made herein based upon knowledge are true, and that all statements made based upon information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

2006/09/21
Date

By T. Sakai
Signature

Takuo SAKAI Takuo Sakai
Typed Name

0397-0441P